Effect of Plant Nutrients on Anthocyanin Content and Yield Component of Black Glutinous Rice Plants

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***Abstract*—**The cultivation of black glutinous rice rich in anthocyanins can provide great benefits to both farmers and consumers. Total anthocyanins content and yield component data of black glutinous rice cultivar (KHHK) grown with the addition of mineral elements (Ca, Mg, Cu, Cr, Fe and Se) under soilless conditions were studied. Ca application increased seed anthocyanins content by three-folds compared to controls. Cu application to rice plants obtained the highest number of grains panicle, panicle length and subsequently high panicle weight. Se application had the largest effect on leaf anthocyanins content, the number of tillers, number of panicles and 100-grain weight. These findings showed that the addition of mineral elements had a positive effect on increasing anthocyanins content in black rice plants and seeds as well as the heightened development of black glutinous rice plant growth.

***Keywords*—**Anthocyanins, black glutinous rice, mineral elements, soilless culture.

I. Introduction

ANTHOCYANINS are coloured pigments often found in nature and are a sub-class of flavonoids [1]. It is the origin of the pigments in black rice. These pigments are one of the most important groups of natural antioxidants and chemopreventive agents found in food [2]. Consumption of foods high in anthocyanins has therefore become popularized due to the various health benefits.

Black rice is a great commodity for the northern regions of Thailand, though the problem lies with the cultivation conditions. Black rice plants are only grown annually; therefore, advantages of increasing rice quality such as anthocyanins content will greatly increase value to the famers [3]. The effort to increase anthocyanins content in black rice plants has sparked interest in this study.

Currently, the knowledge on anthocyanin due to environmental factors is uncertain. Different plants respond differently to the variations in temperature, light, and environmental stress [4]. These stresses can be commonly seen as a mechanism against environmental changes such as the toxicity or deficiency of the available mineral nutrients in the soil [5]. Nutrient availability in soil can influence the effect of flavonoid composition in plant tissue [6] and macronutrients such as nitrogen, potassium and phosphorus have been seen to increase and decrease anthocyanins content in different plant species [7]-[9]. The content of anthocyanins in blackberries was increased when treated with low potassium and high nitrogen fertilizer [4]. Additionally, silicon to barley plants is said to suppress the increase of phenolic content caused by symptoms of excess manganese [10].

Rice research has been focused towards the aroma [11] and post-harvest quality of rice grain [12], but little is known about rice anthocyanin. The study of mineral content in grains and its correlation on grain quality was clearly described by [13]; however, the effect of the addition of mineral elements and its subsequent effect on anthocyanins content should be further analysed. It should be considered that some plants grow best under certain cultivation conditions. With different geographical locations possessing different content of mineral elements in soil, the study of mineral elements such as Ca, Mg, Cu, Cr, Fe and Se on anthocyanins content in black rice plants can assist farmers in northern Thailand to sustainable and efficiently grow black rice plants of high quality. Advanced understanding can provide reference for agricultural purposes for those who wish to increase grain colour characteristics and yield.

II. Methodology

A. Materials and Procedures

Black glutinous rice plants were grown in individual plant pots (n = 3) containing sand and nutrient solution (NPK). Preliminary research of mineral elements at different concentrations was carried out and showed six mineral elements had the most effect on plant growth and yield, therefore were chosen for further investigation in this study. Mineral elements were added to each plant following the concentrations in Table I and were supplied twice a week for a period of 12 weeks. Plant height and number of tillers were recorded weekly from seedling to physical maturity. Leaves were collected at 4 growth stages, namely; tillering, booting, grain-filling and physical maturity. Seeds were collected at physical maturity. Leaves and seeds were air dried to reduce the moisture content (14%) and stored in refrigerator at 4°C until analysis. Yield component data such as panicle weight, panicle length, number of grains per panicle, 100 grain weight were collected and percentage of unfilled grains was calculated.

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B. Extraction of Black Rice Leaf and Seed

For chromatographic analysis, 0.5000 g of leaf and 3.000 g of seeds was extracted with 40 ml of MeOH in 0.1% formic acid (Fâ) and 1 ml of 5 ppm Rhodamine B (RB) as the internal standard. The extract was shaken at 32 °C for 1 hr for leaves and 30 mins for seeds at 280 rpm and filtered using Whatman No.1 filter paper. The collected extract was evaporated using a rotary evaporator set at 40 °C and 60 rpm for approximately 5 min. After evaporation, the crude extract was dissolved in 1 ml MeOH in 0.1% Fâ, filtered through a nylon-membrane filter 0.45 µm and kept at -4°C until analysis. All extraction were performed in triplicates.

TABLE I
CONCENTRATION OF MINERAL ELEMENTS ADDED TO BLACK GLUTINOUS RICE PLANTS

Mineral Element		Concentration (ppm)
Calcium	Ca^{2+}	125.00
Magnesium	Mg^{2+}	100.00
Copper	Cu^{2+}	0.05
Chromium	Cr^{6+}	3.37
Iron	Fe^{3+}	1.50
Selenium	Se^{6+}	0.10

C. Quantification of Anthocyanins

Quantitative analysis of anthocyanins was carried out using HPLC connected to UV-Vis detector. The separation was achieved using Halo C18 (2.1 x 150 mm, 2.7 µm) column and constant temperature of 32°C. The mobile phase consisted of H_2O in 0.5% Fâ, line A, and MeOH, line B. The gradient profile started at 75% solvent A and decreased with a gradient elution to 50% solvent A over 15 min. The gradient continued to decreased to 30% solvent A over 10 min and 0% solvent A over 5 min. The solvent was held at 0% for 3 min and increased to 85% solvent A over 5 min. The solvent was held at 75% for 7 min to achieve a total run time of 45 min. The injector volume was 2µl with flow rate of 0.1 ml/min and UV-Vis detector set at 520 nm. Detected anthocyanins were expressed as µg of cyanidin-3-*O*-glucoside equivalents per g of sample. All analyses were performed in triplicates.

D. Statistical Analysis

Statistical analyses were calculated by one-way analysis of variance (ANOVA) using SPSS software, version 17.0 (SPSS Inc., Chicago, IL). Significant differences were assessed by post hoc Tukey test with level of significance at 95% ($P < 0.05$). Results were expressed as the mean ± standard deviation ($n = 3$).

III. RESULTS AND DISCUSSIONS

A. Effect of Mineral Elements on Plant Height, Tillers and Panicles Number

Black glutinous rice plants grown with the addition of mineral elements showed similar plant height trends ranging from 188 – 204.7 cm with no significant difference at $P < 0.05$. The number of tillers was greater in the Se and control treatment of rice plants than all other mineral treatments (10 ± 2 and 10 ± 1, respectively). The result corresponded with the highest number of panicles (12 ± 1) for Se treatment with significant difference. This suggests Se to have an important role in the development of rice plant stem which could ultimately increase yield. Se has been described to increase root weight of rice plants which indicates its relationship to the rice plant transport system [14]. Se is considered a beneficial micronutrient as it has the ability to mitigate environmental stress such as drought and salinity [15].

The number of tillers was least affected by the addition of Ca and Cu treatments as it had the least number of tillers (7 ± 1 for both mineral elements). Furthermore, the result coincided with the lowest number of panicles (7 ± 1 and 8 ± 1, respectively). The role of Ca and Cu in rice plants relate to the activation of enzymes [16] so it was interesting to see that its application did not correlate to the development of rice plant parts.

B. Effect of Mineral Elements on Yield Component Data

Yield component data such as 100 grain weight, panicle weight, grains per panicle, percentage of unfilled grains, panicles per plant and panicle length are compared in Table II. Se and Mg treatments gave the highest 100 grain weight (2.59 ± 0.06 g and 2.58 ± 0.03 g, respectively) with significant difference. However, results for Mg did not correspond with panicle weight as Mg showed the lowest panicle weight (2.83 ± 0.13 g) in comparison to all other treatments. Furthermore, Mg correlated with a low number of grains per panicles (169 ± 3) and relatively high percentage of unfilled grains (26 ± 2%). This occurrence is normal to rice plants as the weight of panicle axis and branches as well as the length of panicles itself can vary, resulting in a relatively low or high weight. This can be seen in Cu treated plants as the panicle weight (4.75 ± 0.17 g) was significantly higher than all other treatments whilst the average weight of 100 grains was around 2.54 ± 0.02 g. Cu application also gave the highest number of grains per panicle (267 ± 9), longest panicle length (27.9 ± 0.6) though a relatively high percentage of unfilled grains (26 ± 2%) suggesting that the development of panicle branches was much greater than the development of grain quality. Additionally, Se treated rice plants obtained the shortest panicle length and relatively low number of grains per panicles but was able to achieve the highest 100 grain weight illustrating the ability of Se to promote the contents of proteins and carbohydrate in rice grains.

C. Effect of Mineral Elements on Anthocyanins Content

The total anthocyanins content was quantified by HPLC-UV and results are shown in Fig. 1. The variation of mineral content affected anthocyanins content of both leaf and seed of black glutinous rice plants. The tillering stage showed the highest content of leaf anthocyanins, 256.7 µg/g, arising from Se treatment. A drastic decrease (roughly 80%) in anthocyanins content at the booting stage was observed in all treatments. Se was the only treatment to exhibit a gradual increase in leaf anthocyanins content after the booting stage, though the content of anthocyanins in seeds appeared to be one of the lowest (532.9 ± 31.5 µg/g) following iron (453.3 ±

12.3 µg/g). Se was reported to assist the biosynthesis of chlorophyll and improve photosynthetic activity of plants [17] which may be related to the hindrance of anthocyanins biosynthesis [18].

TABLE II
YIELD COMPONENT DATA OF MINERAL ELEMENT TREATMENT

Treatment	100 grain weight (g)	Panicle Weight (g)	Grain/ Panicle	% unfilled grains	Panicle Length (cm)
Se	2.59 ± 0.06 a	3.71 ± 0.17 b	197 ± 8 d	27.30 ± 2.08 ab	24.13 ± 1.21 b
Fe	2.57 ± 0.01 ab	3.15 ± 0.13 c	206 ± 8 cd	14.70 ± 1.53 cd	25.57 ± 0.51 ab
Cr	2.54 ± 0.01 ab	3.11 ± 0.14 c	229 ± 6 b	30.70 ± 2.08a	26.53 ± 0.81 a
Cu	2.54 ± 0.02 ab	4.75 ± 0.17 a	267 ± 9 a	26.00 ± 1.00 b	27.87 ± 0.61 a
Mg	2.58 ± 0.03 a	2.83 ± 0.13 c	169 ± 3 e	26.00 ± 1.73 b	25.93 ± 1.25 ab
Ca	2.50 ± 0.02 b	3.14 ± 0.16 c	235 ± 5 b	12.70 ± 0.58 d	27.47 ± 0.81 a
Control	2.54 ± 0.01 ab	3.91 ± 0.04 b	219 ± 3 bc	18.70 ± 1.15 c	26.20 ± 0.44 ab

Values expressed as the mean ± standard deviation derived from triplicate (n =3) analysis of a single sample. Significant differences (P < 0.05) within the same column are represent by letters.

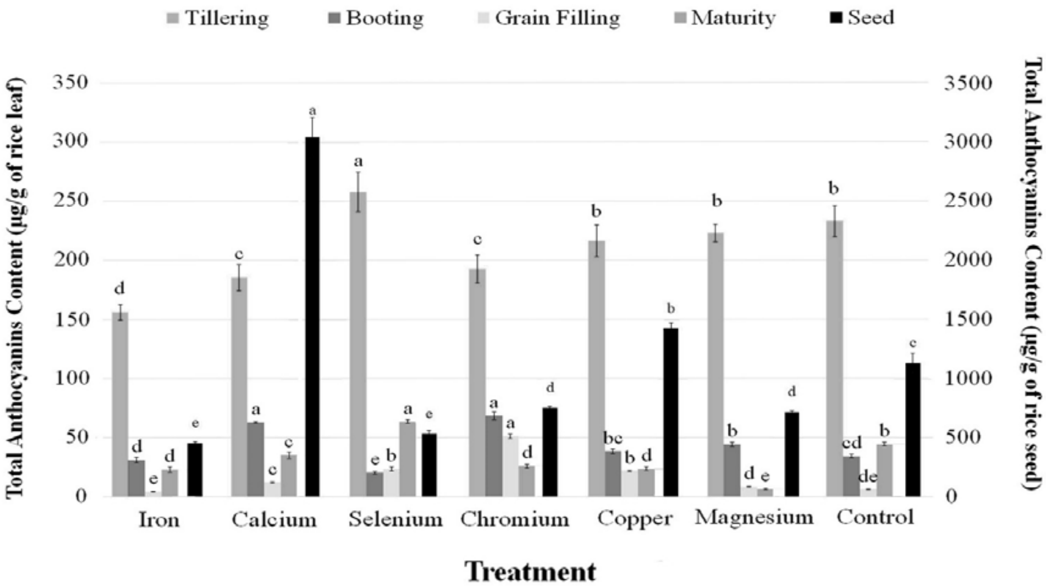

Fig. 1 Total anthocyanins content of black rice leaf and seed at the tillering, booting, grain filling and physical maturity stage (Error bars represent standard deviation of sample (n = 3). Significant differences (P < 0.05) within the same column are represented by superscript letters. Total anthocyanins expressed as C3G equivalent)

A progressive decrease in leaf anthocyanins was seen in Cr treated rice plants from the tillering stage through to the physical maturity stage. The addition of Cr at 3.37 ppm obtained black rice plants with the highest anthocyanins content at the booting (68.2 ± 3.1 µg/g) and grain-filling (51.2 ± 4.5 µg/g) stage but was surpassed by Se treatment at physical maturity. In seeds, the total anthocyanins content of Cr treatment did not exceed that of the control. Cr in plants play an important role in stimulating the translocation of minerals nutrients which could suggest its minor role contributing to the accumulation of anthocyanins in rice plants [19].

Quantification of anthocyanins in seeds of KHHK showed that the addition of Ca markedly increased the content of anthocyanins by three folds in comparison to the control. It was interesting to notice that Ca had the lowest percentage of unfilled grain, suggesting Ca to have an important role in the development of individual rice grains. The application of Ca fertilizer at 125 ppm to black rice paddy could help to increase total anthocyanins content of black glutinous rice. Ca has been seen to increase anthocyanin expression on grape skin as Ca treatments increased the abundance of soluble sugar, a basic component required for anthocyanins biosynthesis [20].

Anthocyanins content was least effected by Fe treatments with significant difference. Black glutinous rice plants with the addition of Fe gave a low contents of total anthocyanins in all developmental stages of leaves and seeds. This suggests that Fe may not be suitable for the improvement of colour trait and rice plant development of black rice. Soils rich in Fe should be taken into consideration for black glutinous rice cultivation.

IV. CONCLUSION

Mineral elements contributed differently to the morphological traits of black rice plants as well as total anthocyanins content in the different growth stages of black glutinous rice cultivation. Se was seen to increase leaf anthocyanins at the tillering and physical maturity stage whilst Cr was seen to increase leaf anthocyanins at the booting and grain-filling stage. Ca was seen to increase anthocyanins content of seeds and contributed to a difference by three folds in comparison to the control. Ca had the lowest percentage of unfilled grains implicating its importance for high grain quality. Se contributed positively to the development of rice plant growth more than the grain traits. Future study on the effects of co-interaction of mineral elements should be investigated.

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